


Complete Genome Sequences of Three Border Disease Virus Strains of the Same Subgenotype, BDSwiss, Isolated from Sheep, Cattle, and Pigs in Switzerland

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ABSTRACT We report here the complete genome sequences of three border disease virus (BDV) strains of the same subgenotype isolated in Switzerland from a sheep, a cow, and a pig, respectively. This is the first report of full-length sequences of a tentatively new subgenotype isolated from three different species of cloven-hoofed farm animals.

Border disease virus (BDV) belongs to the genus *Pestivirus*, which includes important animal pathogens, such as bovine viral diarrhea virus (BVDV) and classical swine fever virus (CSFV). BDV occurs worldwide (1, 2), and several subgenotypes have been described to date (3, 4). BDV infections occur mainly in sheep and goats but also in cattle, pigs, and wild even-toed ungulates (2). We describe here the full-length sequences of three BDV strains isolated in Switzerland from a sheep, a cow, and a pig. They form a new, yet unclassified, BDV subgenotype. The first isolates of this type were isolated in 2006 in Switzerland and provisionally named BD Switzerland (5) or BDSwiss (6–8) or remained unclassified (4, 9, 10), and partial sequences (5′ untranslated region [UTR], N^{pro}) were deposited in GenBank (accession no. JQ994199, JQ994200 GU244490, and GU244489). Recently, BD viruses of this subgenotype were detected in goats and chamois in Italy and were tentatively labeled BDV-8 (11, 12).

Samples R4785/06 (also named CH-BD4), R9336/11, and BD35-15 were obtained from a female white alpine sheep in 2006, a crossbreed (Braunvieh × Limousin) male cow in 2011 (7), and a female domestic pig in a zoo, respectively. Viruses from blood of sheep and cattle were isolated on bovine turbinate cells, whereas SK6 cells were used for porcine blood. RNA was isolated using the QIAamp viral RNA minikit (Qiagen AG, Hombrechtikon, Switzerland), and reverse transcription-PCR (RT-PCR) (Qiagen OneStep RT-PCR kit) was performed according to the manufacturer's instructions. PCR fragments were purified with the QIAquick PCR purification kit (Qiagen). DNA-Sanger cycle sequencing with BigDye Terminator chemistry (version 3.1) and capillary electrophoresis (ABI 3730xl DNA analyzer; Applied Biosystems) were performed at Microsynth (Balgach, Switzerland). The 3′ ends were determined by a simplified protocol for rapid amplification of cDNA ends (RACE) (13) with direct sequencing of the amplification products or by cloning into pCRTM 4-TOPO (Invitrogen). The 5′ ends were determined by a 5′ RACE kit (Invitrogen or Roche). The electropherograms were assembled with SeqMan II version 5.01 (DNASTar, Inc., Madison, WI), and sequences were analyzed with Clone Manager 9 professional edition (Scientific & Educational Software, Cary, NC) and the MEGA program, version 6 (14).

The complete genomes of the isolates R4785/06, R9336/11, and BD35-15 comprise 12,318, 12,311, and 12,309 nucleotides (nt), with 5′ UTRs of 369, 377, and 375 nt and

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3' UTRs of 261, 246, and 246 nt, respectively. All polyprotein-coding sequences are 11,685 nt long and code for 3,895 amino acids (aa). The bovine and porcine isolates are more similar to each other (99% nt, 99% aa) than to the ovine strain (86% nt, 92% aa). In the N^{pro} genomic region, the sequences of the strains reported recently from Italy (11, 12) match 82 to 92% (nt) and 86 to 93% (aa) to the corresponding regions in our strains.

This is the first report of complete consensus sequences of three strains of the same BDV subgenotype, BDSwiss (later also named BDV-8), obtained from a sheep, a cow, and a pig in Switzerland, which will further assist investigations on the epidemiology and evolution of pestiviruses in different host species.

Accession number(s). The complete sequences of the isolates R4785/06, R9336/11, and BD35-15 have been deposited in GenBank under the accession no. [MF102260](#), [MF102261](#), and [MF102262](#), respectively.

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REFERENCES

- Strong R, La Rocca SA, Ibata G, Sandvik T. 2010. Antigenic and genetic characterisation of border disease viruses isolated from UK cattle. *Vet Microbiol* 141:208–215. <https://doi.org/10.1016/j.vetmic.2009.09.010>.
- Schweizer M, Peterhans E. 2014. Pestiviruses. *Annu Rev Anim Biosci* 2:141–163. <https://doi.org/10.1146/annurev-animal-022513-114209>.
- Giammarioli M, Rossi E, Casciari C, Bazzucchi M, Torresi C, De Mia GM. 2015. Genetic characterization of border disease virus (BDV) isolates from small ruminants in Italy. *Virus Genes* 50:321–324. <https://doi.org/10.1007/s11262-014-1165-6>.
- Lešková V, Jacková A, Vlasáková M, Vilček S. 2013. Genetic characterization of a border disease virus isolate originating from Slovakia. *Acta Virol* 57:17–25. https://doi.org/10.4149/av_2013_01_17.
- Peterhans E, Bachofen C, Stalder HP, Schweizer M. 2010. Cytopathic bovine viral diarrhoea viruses (BVDV): emerging pestiviruses doomed to extinction. *Vet Res* 41:44. <https://doi.org/10.1051/vetres/2010016>.
- Casaubon J, Vogt HR, Stalder H, Hug C, Ryser-Degiorgis MP. 2012. Bovine viral diarrhoea virus in free-ranging wild ruminants in Switzerland: low prevalence of infection despite regular interactions with domestic livestock. *BMC Vet Res* 8:204. <https://doi.org/10.1186/1746-6148-8-204>.
- Braun U, Hilbe M, Janett F, Hässig M, Zanoni R, Frei S, Schweizer M. 2015. Transmission of border disease virus from a persistently infected calf to seronegative heifers in early pregnancy. *BMC Vet Res* 11:43. <https://doi.org/10.1186/s12917-014-0275-7>.
- Kaiser V, Nebel L, Schüpbach-Regula G, Zanoni RG, Schweizer M. 2017. Influence of border disease virus (BDV) on serological surveillance within the bovine virus diarrhoea (BVD) eradication program in Switzerland. *BMC Vet Res* 13:21. <https://doi.org/10.1186/s12917-016-0932-0>.
- Bachofen C, Stalder HP, Vogt HR, Wegmüller M, Schweizer M, Zanoni R, Peterhans E. 2013. Bovine viral diarrhoea (BVD): from biology to control. *Berl Münch Tierarztl Wochenschr* 126:452–461. (In German.)
- Braun U, Reichle SF, Reichert C, Hässig M, Stalder HP, Bachofen C, Peterhans E. 2014. Sheep persistently infected with border disease readily transmit virus to calves seronegative to BVD virus. *Vet Microbiol* 168:98–104. <https://doi.org/10.1016/j.vetmic.2013.11.004>.
- Peletto S, Caruso C, Cerutti F, Modesto P, Zoppi S, Dondo A, Acutis PL, Masoero L. 2016. A new genotype of border disease virus with implications for molecular diagnostics. *Arch Virol* 161:471–477. <https://doi.org/10.1007/s00705-015-2696-4>.
- Caruso C, Peletto S, Cerutti F, Modesto P, Robetto S, Domenis L, Masoero L, Acutis PL. 2017. Evidence of circulation of the novel border disease virus genotype 8 in chamois. *Arch Virol* 162:511–515. <https://doi.org/10.1007/s00705-016-3112-4>.
- Scotto-Lavino E, Du GW, Frohman MA. 2006. 5' End cDNA amplification using classic RACE. *Nat Protoc* 1:2555–2562. <https://doi.org/10.1038/nprot.2006.480>.
- Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S. 2011. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Mol Biol Evol* 28:2731–2739. <https://doi.org/10.1093/molbev/msr121>.